46

HUMAN PAPILLOMAVIRUS TYPE 16 (HPV16) E6/E7-SPECIFIC CYTOTOXIC T LYMPHOCYTES (CTLs) FOR IMMUNOTHERAPY OF HPV-ASSOCIATED MALIGNANCIES

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Vaccines prevent HPV-associated cancer (Ca), but their use as therapy for established Ca has been disappointing. Although the target tumor cells express the viral E6 and E7 antigens (Ag), patients' immune responses against virally infected cells are limited, even after active immunization, likely due to negative environmental cues that block initial tumor cell recognition and subsequent T cell (TC) activation and expansion in vivo. We postulated that ex vivo stimulation of patient TCs in an immunologically favorable milieu would allow us to reactivate tumor-directed CTLs.

We studied 67 patients with HPV+ Ca (16 cervical and 51 oropharyngeal, OPCa). To investigate the presence of HPV16 E6- and E7-specific TCs (HPV-TCs) in blood, we measured the γ-IFN ELISpot responses of TCs stimulated by monocyte-derived dendritic cells (DCs) loaded with pepmixes (peptide libraries of 15-mers overlapping by 11 aa) spanning E6 and E7. Although ~75% of OPCa are HPV16+, we initially found no evidence of E6/E7-reactive T cells in the patients tested. Because HPV-TCs from these patients may be anergized by their tumors, we postulated that potent Ag presenting strategies might be required for reactivation. In other studies, we have found that in vitro stimulation of T cells in the presence of DCs and IL-6, -7, -12 and -15 can induce responses to poorly immunogenic Ag. We therefore tested this approach in patients with HPV+ Ca, and found we could successfully reactivate HPV-TCs in 8/16 cervical and 32/51 OPCa patients.

Given it is difficult to obtain large numbers of DCs, we expanded these HPV-TCs to clinically useful numbers by substituting patient B-cell blasts (BBs) as APCs, which we made by culturing autologous PBMCs with IL-4 on a CD40L+ feeder layer. Stimulation of DC-stimulated HPV-TCs by E6/E7 pepmix-loaded BBs further expanded HPV-TC lines (3.8 ± 1.5×/round), whose phenotype is summarized in the Table. The epitopes recognized by the HPV-TCs mapped to E6 aa 49-71, 77-91 and 125-143, and E7 aa 1-19 and 73-87. These cells achieved dose-dependent specific killing of E6/E7+ target cells (specific lysis up to 45-61% vs. 0-8% in controls, 40:1 E:T ratio). Thus, we have generated true CTLs.

Table. Phenotypic analysis of HPV-specific cell lines

Marker	% ± SD
CD3	97.5 ± 3.4
CD4	36.7 ± 28.2
CD8	49.4 ± 27.0
CD56 (CD3 negative)	1.5 ± 0.9

All cell lines are almost exclusively composed of T cells, with a variable proportion of $CD4^+$ and $CD8^+$ cells, displaying a predominantly effector memory phenotype ($CD45RA^-$, $CD45RO^+$, $CD62L^-$ and $CCR7^-$). There are minimal $CD3^-$ NK cells. Vβ-chain TCR repertoire analysis established polyclonality.

In summary, we have developed a system that allows the robust generation of HPV-directed CTLs from the blood of patients with HPV16+ Ca, and shown that they recognize specific epitopes in tumor-associated Ag. Our lines have the potential to be used for adoptive cellular immunotherapy of HPV+ Ca.

47

NOVEL THERAPY WITH INTERFERON-α IN COMBINATION WITH DONOR LYMPHOCYTE INFUSION FOR HIGH RISK ACUTE LEUKEMIA PATIENTS WHO RELAPSED AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objective: In order to improve the graft versus leukemia (GVL) effect of DLI, we investigated the efficacy and safety of combining interferon-α with DLI (aDLI) in patients with high risk acute leukemia (AL) who relapsed after allo-HSCT, and compared the efficacy, toxicity and leukemia free survival (LFS) of aDLI and traditional donor lymphocyte infusion (tDLI) in our transplantation center.
Methods: Sixteen acute leukemia patients were treated with interferon-α-2b therapy at a dose of 3×10^6U/day subcutaneously for 5 days followed by G-CSF mobilized donor peripheral stem cell infusion. (termed with "aDLI"). The median duration of IFN-α treatment was 17 (5-50) days, and the median $CD3^+$ cells dose was 9.25×10^7/kg ($4\text{-}20\times10^7$/kg). In parallel, we retrospectively analyzed the results of tDLI for 14 AL patients with hematologic relapse post allo-HSCT treated in the same period in our center, and compared the efficacy, toxicity and LFS of tDLI with aDLI.
Results: Patients treated on the aDLI protocol included 9 ALL and 7 AML, with a median age of 26.5 years. Fourteen of 16 patients had high risk AL. The median relapse time was 5.5 (range, 1-25) months post transplant. Salvage chemotherapy was administrated in 7 patients before aDLI, with only 3 patients achieved CR. The overall CR rate for aDLI protocol was 75% (12/16), with CR rate of aDLI alone at 66.7% (6/9). The median time from aDLI to bone marrow CR was 7 (6-14) days, and the median time to molecular CR (MCR) and full donor chemerism (median level was 96.3%) in responsive patients were 2 weeks post DLI. With a median follow-up of 5.5 (range, 1-34) months, 7 patients were alive with durable molecular CR. Two-year LFS was 50%. Treatment related toxicities included episode of fever, GVHD and myelosuppression. The tDLI group had similar demographic characteristics with disease subtypes, transplant and relapse history. Compared to tDLI, the aDLI protocol had higher CR rate (75.0% vs 14.3%, $p = 0.001$), faster response (median time to obtain BM CR were 7 days), and significant better 2-year LFS (50.0% vs 7.1%, $p = 0.05$). Importantly, there was no significant difference between the two groups with respect to the incidence of pancytopenia (53.8% vs 75%, $p>0.05$) and treatment related mortality (18.8% vs 7.1%, $p>0.05$).
Conclusion: IFN-α-2b in combination with DLI appears to induce rapid and durable remissions in high risk acute leukemia patients who relapsed following allo-HSCT, with acceptable treatment-related toxicity.

48

SALVAGE T CELL THERAPY FOR THERAPY RESISTANT VIRAL DISEASES AFTER STEM CELL TRANSPLANTATION

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Epstein-Barr virus (EBV), cytomegalovirus (CMV) and adenoviral reactivations are frequent complications after allogeneic SCT because of a lack of T cell control due to extensive immunosuppression. Cytotoxic T lymphocytes (CTLs) that recognize viral antigens are the most important immune effector mechanism controlling the persistent viral infections.

First-line treatment for viral reactivation is dose reduction of the immunosuppressive drugs and/or anti-viral therapy. For PTLD this is followed by rituximab (anti-CD20 monoclonal antibody). Despite these multiple treatment strategies, the l mortality from drug-resistant viremia after SCT is still considerable. Another treatment approach is adoptive transfer of virus specific CTLs from the donor. The standard method of adoptive T cell immunotherapy is laborious and time-consuming and is often too late to be administrated to the patient.

We have developed a clinical separation protocol for virus specific CTLs based on labeling with multimeric complexes containing recombinant HLA molecules together with virus derived peptides. By combining this labeling technique with a secondary magnetic sorting we have managed to get a high purity of specific CTLs. This high purity diminish the risk of creating GVHD in the recipient even if the adoptive transfer of cells is done in an allogeneic or haplo-identical setting.

We first used this protocol in an 18 year old patient with life-threatening PTLD. The patient developed an EBV associated lymphoma involving lungs, liver and both kidneys and also showed extremely high EBV titers in blood. It was decided to give her EBV specific CTLs from her mother. 2 months after the given EBV specific CTL infusion the EBV associated lymphoma was in complete regression. After this we have further successfully treated seven patients with life threatening viral disease (Adeno, CMV and EBV) with good efficacy. We could see a clinical and immunological

response in six out of eigth patients. In five out of six of these responding patients we have been able to identify infused T cells using chimerism analysis and SNP markers specific for the CTL donor. This method opens up the possibility to rapidly treat patients which are in acute need of T cell therapy and cannot wait for prolonged expansion techniques or cannot tolerate standard treatment regiment.

GVH/GVL

49

EXPRESSION OF α4β7 INTEGRIN ON MEMORY CD8+ T-CELLS IS INCREASED IN PATIENTS AT PRESENTATION OF ACUTE INTESTINAL GRAFT-VS-HOST DISEASE

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Background: Acute intestinal graft-vs.-host disease (GVHD) remains a major source of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). α4β7 integrin is a cell surface molecule thought to mediate specific lymphocyte trafficking to intestinal tissue. Our previous work suggested that upregulation of α4β7 integrin expression on memory CD4+ and CD8+ peripheral blood T-cells after HCT correlated with the future development of intestinal GVHD.

Methods: In this analysis, peripheral blood mononuclear cells were collected from patients at the time of presentation of symptoms of GVHD prior to the initiation of systemic corticosteroids. In total, 27 patient samples were collected prospectively after HCT, which were then retrospectively divided into 3 cohorts: GUT (intestinal GVHD, n = 11), SKIN (cutaneous GVHD, n = 9), and NONE (suggestive symptoms but negative evaluation for GVHD, n = 7). Sample collection ranged from day +23 to day +194 after HCT and was based on symptoms or signs suggestive of GVHD. One patient developed GVHD after donor leukocyte infusion (DLI).

Results: There were no significant differences in baseline characteristics between the groups. Analysis by flow cytometry showed that GUT patients had a significantly higher percentage of α4β7 integrin expressing memory (defined by CD45RO+) peripheral blood CD8+ T-cells (median 7.7%, range 0.5%-28.1%) compared to the SKIN group (2.1%, 0%-3.1%, p = 0.03), the NONE group (1%, 0.2%-2.2%, p = 0.02), or the SKIN and NONE groups combined into one NON-GUT (n = 16) group (1.6%, 0%-3.1%, p = 0.01). Time of sample collection after HCT had no effect on the expression of α4β7 integrin.

Conclusion: These results add to the evidence that upregulation of α4β7 integrin on memory CD8+ T-cells may be involved in lymphocyte trafficking in acute intestinal GVHD and increased expression may be a potential biomarker. More importantly, α4β7 integrin may represent a novel target of treatment and prophylaxis of acute intestinal GVHD after HCT with specific monoclonal antibodies already in development for the treatment of inflammatory bowel disease. Analysis with a larger number of samples is planned to confirm these results.

50

INFUSION OF SYNGENEIC APOPTOTIC CELLS PRIOR TO BONE MARROW TRANSPLANTATION DECREASES TCELL PROLIFERATION AND INCREASES SURVIVAL

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To prevent graft versus host disease (GVHD) the graft has to be rendered tolerant to avoid alloreactivity towards the host. One promising approach to induce tolerance is to administer apoptotic cells to the host. Apoptosis can be induced by extracorporeal photopheresis (ECP), a therapy based on exposure of cells to photoactivable 8-methoxypsoralen (8MPO) and UVA irradiation. The effect of ECP treatment is at least in part, due to alteration of dendritic cell (DC) maturation, leading to reduced Tcell activation. In contrast to clinical practice where ECP is only applied to established GVHD, we have developed a pre-emptive murine model with the aim of inhibiting the initiation phase of acute GVHD.

Methods: Host apoptotic splenocytes were infused into MHC matched or mismatched recipients 5 or 2 days prior to lethal irradiation and BMT. Apoptotis of cells was generated by 8-MPO and UVA light (UVAR light set, Therakos). Mice were followed for GVHD score and survival.

Results: ECP-treated mice survived longer both across major (median survival 51 versus 26 days, $p<0.0001$) and minor (median survival 56.5 versus 9 days, $p<0.002$) MHC barriers. Importantly, even though ECP treatment promoted tolerance against the host it did not impact the graft versus tumor effect in a B-cell lymphoma model. In vivo experiments using bioluminescent imaging showed reduced CD4+Tcell proliferation during the initiation phase of GVHD (day+4) in ECP-treated mice compared to mice receiving Tcon only. The same result was found in CFSE labeled Tcells re-isolated on day+4. FACS-sorted DC incubated with apoptotic cells for 2 days in vitro showed a diminished capability to induce Tcell proliferation. Re-isolation experiments on day+3 confirmed lower expression of homing (P-Selectin, α4β7) and activation (CD44, CD69) markers on donor Tcells in ECP treated groups, indicating slower trafficking to GvHD target organs. Furthermore, at day+7 ECP-treated groups showed increased FoxP3-expressing host Tregs in comparison to control groups whereas donor Treg were similar in both groups.

In summary, ECP treatment prior to transplantation led to a significant survival benefit with no impact on graft versus tumor effect; reduced CD4+ Tcell proliferation; delayed expression of homing and activation markers during the initiation phase of GVHD and an increase in host Treg. Overall our work suggests that pre-emptive ECP of host cells prior to BMT may be a clinically relevant strategy to prevent acute GVHD.

51

LOW LEVELS OF 25-HYDROXYVITAMIN D PRIOR TO ALLOGENEIC TRANSPLANTATION CORRELATE WITH THE DEVELOPMENT OF CHRONIC GRAFT-VERSUS-HOST DISEASE

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Graft-versus-host disease (GVHD) remains a significant cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation. GVHD is mediated by the activation and expansion of donor derived alloreactive lymphocytes stimulated in part by residual host dendritic cells (DCs) and donor DCs with cross presentation of alloantigens. Vitamin D is a hormone essential for calcium homeostasis and exhibits potent immunomodulatory characteristics. We have demonstrated that vitamin D exposure inhibits DC maturation and DC mediated stimulation of allogeneic T cells. As such, we hypothesized that vitamin D deficiency may result in the enhanced capacity of DCs to induce GVHD. In this study, we performed a retrospective case-control study to evaluate whether monohydroxyvitamin D levels measured in serum samples obtained from patients prior to allogeneic transplantation were predictive of the risk for GVHD. Fifty-three patients aged 18-60 who underwent a myeloablative matched related or matched unrelated donor transplant between December 2000 and December 2009 were included in the analysis. 25-OH vitamin D concentrations were measured by an ELISA assay prior to transplantation. The median 25-OH vitamin D level was 21.9 ng/mL (range 7.8-45.7). Patients were divided into two cohorts based on a vitamin D level: < 25 ng/mL (32 patients) or ≥ 25 ng/mL (21 patients). Remarkably, the cumulative incidence (CI) of chronic GVHD (cGVHD) at 2 years in patients with 25-OH vitamin D <25 was 63.8%, compared to 23.8% in patients with vitamin D levels ≥ 25 ($p = 0.009$). Similarly the 2 year CI of extensive cGVHD was significantly greater in patients with vitamin D levels <25 compared to those with ≥ 25 (54.5% versus 14.3%, $p = 0.005$). This result is consistent in multivariable competing risks model for which patient and transplant related factors mentioned above were adjusted (HR 5.26, $p = 0.02$). Three-year progression-free survival and overall survival were similar between the two groups (51% vs. 47%, $p = 0.61$; and 53% vs. 50%, $p = 0.57$ respectively). In summary, vitamin D deficiency prior to allogeneic transplantation is associated with a significantly increased risk of chronic GVHD. A prospective clinical trial evaluating the use of vitamin D for the prevention of chronic GVHD is planned.